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14. ABSTRACT

The goals of our research are to understand the mechanisms of recognition of ovarian cancer by the immune system, to develop peripheral and tissue-based biomarkers predictive of response to anti-PD-1-based therapeutic approaches, and to identify/develop new combinatorial therapeutic strategies based on systemic and locoregional immune targeting approaches. Our research over the past year has generated several findings. First, we characterized peripheral blood populations in patients with ovarian cancer treated with immune checkpoint blockade and found that these treatments are associated with upregulation of additional immune checkpoints such as LAG-3, providing rationale for further combinations. Secondly, we have found that tumors exhibiting low mutational burden can still exhibit high levels of T cell infiltration, which is generating new research directions, prompting us to examine the interaction between the tumor-intrinsic driver pathways and the immune system. Third, we have initiated animal studies using intratumoral modulation with recombinant oncolytic Newcastle Disease Virus (NDV). While looking for the most effective combination of recombinant NDV with systemic immunomodulatory agents, we found that pre-existing immunity to the virus potentiates, rather than inhibits its therapeutic efficacy. This finding indicates that patients undergoing such treatments may benefit from therapy even if they develop neutralizing anti-viral antibodies.

15. SUBJECT TERMS
Ovarian cancer, immunotherapy, oncolytic virus, checkpoint inhibitor, T cell

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1. **INTRODUCTION:**

In epithelial ovarian cancer (EOC), immunotherapies with PD-1/PD-L1 blocking drugs have been evaluated in preliminary trials with promising response rates. Despite these significant clinical advances, the benefit afforded by PD-1/PD-L1 blockade in ovarian cancer has not been universal, calling for identification of mechanisms of response and resistance to these drugs and development of novel combinatorial approaches. The major goals of my research are to explore the mechanisms of response/resistance in patients with EOC treated with immune checkpoint blockade and to develop novel immunotherapeutic combinations using locoregional and systemic treatment strategies in animal tumor models. The overall goal of the proposal is to eventually develop a clinical trial that would incorporate the findings from patients and pre-clinical models to address the mechanisms of PD-1 resistance in ovarian cancer patients.

2. **KEYWORDS:**

Ovarian cancer, immunotherapy, oncolytic virus, immune checkpoint blockade, tumor microenvironment.

3. **ACCOMPLISHMENTS:**

Specific Aim 1: Identify the immune-activating and compensatory immunosuppressive mechanisms upregulated in response to PD-1 and PD-L1 blockade in human ovarian cancer.

Proposed and achieved milestones for Aim 1 for year 1 include:
- Submission and approval of IRB protocol by HRPO
- Collection of the tumor samples necessary for analysis
- Initiation of the analysis of tumor samples, as specified in Aim 1

Specific Aim 2: Evaluate therapeutic strategies targeting mechanisms of immune resistance and activation through systemic and locoregional immunomodulation

Proposed and achieved milestones for Aim 2 for year 1 include:
- Submission and approval of the IACUC protocol by ACURO
- Initiation of the animal studies using ID8, MB49, and B16-F10 tumor models

What was accomplished under these goals?

Specific Aim 1: Identify the immune-activating and compensatory immunosuppressive mechanisms upregulated in response to PD-1 and PD-L1 blockade in human ovarian cancer.

A. Peripheral blood studies

In a cohort of 15 ovarian cancer patients treated with nivolumab or nivolumab and ipilimumab we have analyzed the changes in the peripheral blood subsets by flow cytometry using the staining panels characterizing the markers of T cell activation, T cell inhibition, and the levels of myeloid-derived suppressor cells. In addition, RNA collected from PBMC's was analyzed using the Nanostring PanCancer Immune profiling platform. In this cohort of 15 patients, only 3 patients demonstrated clinical benefit, with one additional patient with disease stabilization after initial progression. Patients treated with dual checkpoint blockade exhibited increased levels of activation and proliferation markers as opposed to the PD-1 blockade alone. There was no consistent correlation between treatment and peripheral blood MDSC levels. Interestingly, in all patients, therapy with PD-1 and PD-1/CTLA-4 blockade resulted in sustained upregulation of LAG-3 and TIM-3 on T cells, additional two immune checkpoints that have been demonstrated to be responsible for T cell exhaustion (Fig. 1). This finding provides a need for validation of this marker in a larger cohort of patients and provides a rationale for dual blockade of LAG-3 and PD-1 in patients with ovarian cancer. To broaden the number of potential markers, we have explored Nanostring platform for analysis of immune-related genes in peripheral blood. While this study demonstrated transcriptional pharmacodynamics changes in the blood in response to PD-1 or PD-1/CTLA-4 blockade, it failed to identify any specific markers associated with response to the immune checkpoint inhibitors. Overall, these studies suggest that bulk analysis of cells in peripheral blood, either by flow cytometric or by

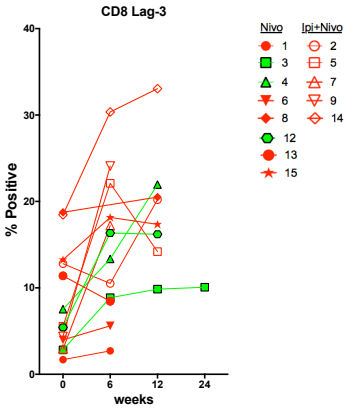


Figure 1. Expression of LAG-3 immune checkpoint in PBMC CD8+ cells in response to immunomodulatory antibody therapy. Green samples denote patients with clinical benefit.

transcriptional profiling has poor sensitivity for identification of markers that could be predictive of response to immunotherapy in ovarian cancer patients.

Ongoing studies

Recent studies have demonstrated that expansion of intratumoral T cell clones in the peripheral blood could be predictive of clinical benefit in response to checkpoint blockade in melanoma and bladder cancer. To build on these findings, we are planning to establish a collaboration with Atreca, whose technology uses single-cell platform to identify individual activated T cells and B cells and the sequences of their corresponding T cell and B cell receptors in the peripheral blood. Using this technology, we are thus able to focus not just on T cell activation markers, but to match these markers to specific TCR clonotypes. By further matching these TCR clonotypes to TCRs identified in tumors, we will be able to more accurately track the fate of specific T cells within the peripheral blood. Aside from tissue studies, we have in addition characterized some clinical features distinguishing patients that progress early on immunotherapy and submitted these findings as an abstract for the SGO 2018 meeting (see Appendix).

B. Tissue studies

Our tissue-based studies to date have focused on transcriptional analysis of immune-related genes in tumors and on microscopic analyses of tumor immune architecture. To optimize these methods, while awaiting the tissue samples from the patients treated with immunomodulatory antibody therapy, we have partnered with Dr. Douglas Levine to analyze the tumor immune microenvironment of the small cell carcinoma of the ovary hypercalcemic type (SCCOHT), a rare ovarian cancer characterized by genetic alterations in SMARCA4 gene, a component of the SWI/SNF chromatin remodeling complex. These tumors otherwise are characterized by a very low mutational burden, dampening an enthusiasm for immunotherapy in these patients. Unexpectedly for a low mutational burden tumor, these tumors exhibited robust T cell infiltration and PD-L1 expression (Figure 2). Transcriptional profiling of these tumors revealed expression of high levels of genes related to T cell function and cytotoxicity (Figure 2). These findings thus provide a rationale for evaluation of PD-1-targeting strategies in SCCOHT. Indeed, we identified 4 patients that received nivolumab and either responded or had durable clinical benefit from therapy. These results also suggest that there may be a link between alteration in tumor chromatin remodeling pathways and tumor immune recognition and we are currently further pursuing these studies. The findings from this part of the project have been submitted for publication at JNCI and the manuscript is currently under secondary review.

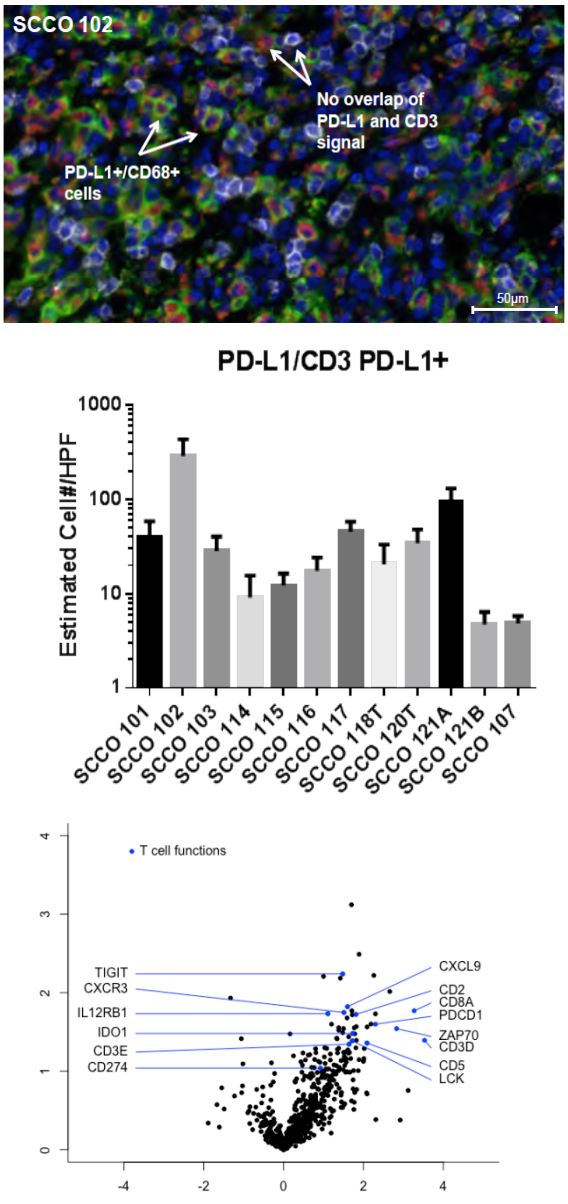


Figure 2. SCCOHT tumors exhibit T cell infiltration and PD-L1 expression. Representative IF image (top) and quantification (middle) are shown. Bottom: transcriptional analysis of PD-L1 high vs. PD-L1 low tumors using Nanosting PanCancer immune profiling.

Specific Aim 2: Evaluate therapeutic strategies targeting mechanisms of immune resistance and activation through systemic and locoregional immunomodulation

This aim is based on the technology being developed in the lab, using genetically-engineered oncolytic Newcastle Disease Virus (NDV) in combination with systemic agents targeting mechanisms of immune resistance. In these studies, we employed syngeneic B16 melanoma and ID8 ovarian carcinoma models. Our studies during the Cycle for Survival grant period have generated findings that have enhanced our understanding of the mechanisms of NDV-based immunotherapy and has generated rationale for clinical studies.

1. Pre-existing immunity to oncolytic virus potentiates its immunotherapeutic efficacy.

Anti-viral immunity presents a major hurdle for systemically-administered oncolytic viruses (OV). Intratumoral OV therapy has a potential to overcome this problem through activation of anti-tumor immune response, with local and abscopal effects. However, the effects of anti-viral immunity in such a setting are still not well defined. Using NDV as a

model, we explored the effects of pre-existing anti-viral immunity on therapeutic efficacy in syngeneic mouse tumor models. Unexpectedly, we find that while pre-existing immunity to NDV limits its replication in tumors, tumor clearance, abscopal effects and survival are not compromised and, on the contrary, are superior in NDV-immunized mice, both in a setting of NDV monotherapy (Figure 3) and in combination with systemic CTLA-4 blockade. These findings demonstrate that pre-existing immunity to NDV may increase its therapeutic efficacy through potentiation of systemic anti-tumor immunity, which provides clinical rationale for repeated therapeutic dosing and prompts investigation of such effects with other OVs. The results of these studies have been submitted for publication and the manuscript is currently under review.

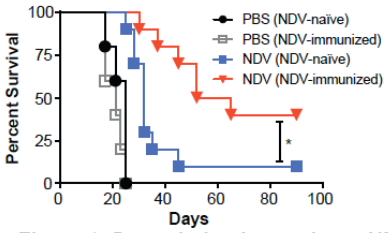


Figure 3. Pre-existing immunity to NDV potentiates its efficacy. NDV-immunized or control animals were challenged with B16-F10 melanoma and subsequently treated with intratumoral NDV injection.

2. **Upregulation of PD-L1 in tumor microenvironment is a resistance mechanism for oncolytic virus immunotherapy**

Intralesional therapy with oncolytic viruses leads to activation of local and systemic immune pathways, which may present targets for further combinations. Using human tumor histocultures as well as syngeneic tumor models treated with NDV we identified a range of immune targets upregulated with treatment. Despite the tumor effector T lymphocyte infiltration in response to NDV, there is ongoing inhibition through PD-L1, acting as an early and late adaptive immune resistance mechanism to type I IFN response and T cell infiltration, respectively. Systemic therapeutic targeting of PD-1 or PD-L1 in combination with intratumoral NDV resulted in rejection of both treated and distant tumors. These findings provide implications fo r timing of PD-1/PD-L1 blockade in conjunction with OV therapy and highlight that understanding of adaptive mechanism s of immune resistance to specific OV will be important for rational design of combinatorial approaches with these agents. Another manuscript describing these findings is currently under review.

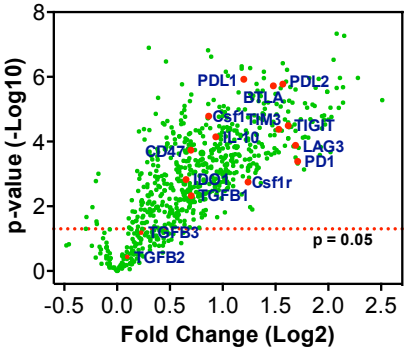


Figure 4. NDV upregulates a range of immune inhibitory molecules in B16 melanoma. Immune-related gene expression in NDV-injected tumors analyzed using Nanostring PanCancer immune profiling panel.

We have expanded on these studies by targeting additional mechanisms of immune inhibition through addition of other immunotherapy agents that are currently in clinical development, including drugs targeting IDO, CSF-1R, and phosphatidylserine. Surprisingly, despite upregulation of these mechanisms with NDV and NDV+anti-PD-1 therapy, targeting of these additional mechanisms did not further enhance efficacy. Furthermore, depletion of tumor-associated macrophages using this strategy was detrimental to therapeutic efficacy, suggesting that macrophage infiltration observed with NDV therapy may be an active player in anti-tumor activity, rather than a negative feedback mechanism.

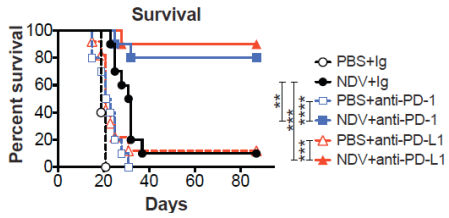


Figure 5. Combination of intratumoral NDV with systemic PD-1 or PD-L1 blockade potentiates therapeutic efficacy against local and distant tumors.

3. **Intratumoral modulation of the inducible costimulator (ICOS) by recombinant oncolytic virus promotes systemic anti-tumor immunity**

Emerging data suggest that locoregional cancer therapeutic approaches with oncolytic viruses can lead to systemic anti-tumor immunity, although the appropriate targets for intratumoral immunomodulation using this strategy are not yet known. We find that intratumoral therapy with NDV, in addition to activation of innate immunity, upregulates the expression of T cell co-stimulatory receptors, with the inducible costimulator (ICOS) being most notable (Figure 6). To explore ICOS as a direct target in the tumor, we engineered a recombinant NDV expressing ICOS ligand (NDV-ICOSL). In the bilateral flank tumor models, intratumoral administration of NDV-ICOSL resulted in enhanced infiltration with activated T cells in both virus-injected and distant tumors, and led to effective rejection of both tumors when used in combination

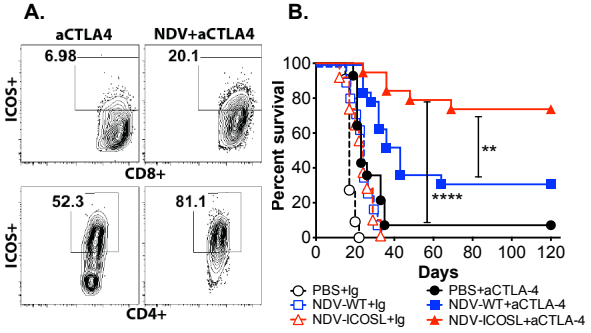


Figure 6. NDV-ICOSL in combination with anti-CTLA-4 leads to sustained distant B16 melanoma remissions. (A) Upregulation of ICOS on TILs. (B) Long-term survival in the animals treated with combination of intratumoral NDV and systemic anti-CTLA-4 according to the schedule in 3A.

with systemic CTLA-4 blockade (Figure 6). These findings highlight that intratumoral immunomodulation with an oncolytic virus expressing a rationally-selected immune ligand can be an effective strategy to drive systemic efficacy of immune checkpoint blockade. These findings were published in Nature Communications in 2/2017. We have taken these findings further and have screened a panel of NDV's expressing various immune stimulators, with several preliminary candidates identified for further testing.

What opportunities for training and professional development has the project provided?
The project provided training opportunity for Mr. Jacob Ricca, who was a research technician in the laboratory. As a result of the project, Jacob has developed a broad set of laboratory skills and was able to present his findings as an oral abstract in the 2016 Annual Meeting of the Society for Immunotherapy of Cancer (SITC). The results of the findings were submitted for a publication, on which Jacob is a first author. He now went on to pursue training in medical school.
The project has also provided further opportunities for professional development for Dmitriy Zamarin, PI of the project. Dr. Zamarin has presented some of the results of the findings as a keynote speaker at the 2017 BioCanRx Immunotherapy Summit in June of 2017 in Canada. He has attended NRG Oncology Group conference in June of 2017 and presented a biomarker plan for protocol NRG-GY003 (nivolumab vs. nivolumab+ipilimumab in patients with platinum resistant ovarian cancer). He will lead the biomarker effort for the entire multi-center study. Dr. Zamarin has worked closely with his mentors Dr. Wolchok and Dr. Aghajanian to review his research progress and career planning. Dr. Zamarin has in addition composed an investigator-sponsored clinical protocol using intraperitoneal oncolytic virus in combination with systemic PD-L1 blockade in patients with ovarian cancer. The protocol is due to open next month.

How were the results disseminated to communities of interest?

2 manuscripts are currently in submission.

2 abstracts have been submitted to the Society for Gynecologic Oncology 2018 meeting

What do you plan to do during the next reporting period to accomplish the goals?
For the next reporting period, we will focus closely on the studies proposed in the Aim 1. Specifically, since all of the proposed samples have been obtained, our next goal will involve analyses of the archival tissue samples, which will include whole exome sequencing, gene expression profiling, multi-parameter microscopy, and TCR profiling.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

1. Currently, the response to immunotherapy with immune checkpoint blocking antibodies such as anti-PD-1 is thought to rely heavily on the tumor mutational burden (i.e. tumors with more mutations appear to be more immunogenic and more responsive). Our data from the studies of small cell ovarian cancer of hypercalcemic type (SCCOHT) reveal that even in the absence of significant number of mutations, tumors can still be immunogenic and recruit T cells. This finding is likely to generate new research directions in ovarian cancer, looking at other mechanisms by which the immune system is able to recognize tumors.
2. Based on the preclinical findings with combination of oncolytic virus therapy and anti-PD-1 therapy, we are opening a trial to evaluate this finding in ovarian cancer patients.

What was the impact on other disciplines?
The prevailing hypothesis in the field of oncolytic viruses suggests that prior immunity to oncolytic viruses can compromise therapeutic efficacy by neutralizing the virus and preventing the infection of tumor cells. Our findings using oncolytic virus administered intratumorally suggest otherwise: it appears that prior immunity to the virus actually enhances the anti-tumor immune effect.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

None

Actual or anticipated problems or delays and actions or plans to resolve them
We have been delayed in performing tissue-based analyses from the ovarian cancer patients treated with immune checkpoint blockade due to lack of tissue availability and response rate that has been lower than expected, making it challenging to perform robust comparisons between responders and nonresponders. We have now been able to collect pre-treatment core biopsy tissue blocks from 10 responder patients and 20 non-responder patients from these studies. These numbers should be sufficient for comparison of biomarkers predicting response/resistance in these patients.

In animal experiments, we have found that the ID8 model is suboptimal for the proposed studies. At the moment, we are using other syngeneic tumor cell lines to perform the proposed experiments. In addition, I have reached out to several investigators who have other syngeneic ovarian cancer cell lines and am awaiting to start these experiments.

Changes that had a significant impact on expenditures

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects
The protocol was amended to include additional personnel. The amendment was approved by the IRB. Continuing review report as well as the amendment was submitted to the agency. Amendment approval: 08/09/2017. CRR approval: 7/31/17.

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

6. **PRODUCTS:**

Publications, conference papers, and presentations

Journal publications.

-

1. Ricca J, Oseledchyk A, Merghoub T, Wolchok JD, **Zamarin D.** Pre-existing immunity to oncolytic virus potentiates its therapeutic efficacy. *Under Review*

2. **Zamarin D,** Ricca J, Oseledchyk A, Merghoub T, Wolchok JD. Upregulation of PD-L1 in tumor microenvironment is a resistance mechanism for oncolytic virus immunotherapy. *Under Review*

3. Jelinic P, Ricca J, Van Oudenhove E, Olvera N, Bisognas M, Levine DA, **Zamarin D.** Immune-active microenvironment in SCCOHT: rationale for therapy with immune checkpoint blockade. *Under Review*

i. **Other publications, conference papers, and presentations.**

1. Ricca J., Oseledchyk A., **Zamarin D.** Locoregional therapeutics: using one's own tumor as a vaccine. *Journal of Targeted Therapies.* (April 2017) *In Press.*

2.

4. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

a. **What individuals have worked on the project?**

i.

Name:	*Dmitriy Zamarin*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Zamarin is the PI of the overall project. He performed teaching of laboratory techniques for the laboratory members, and was involved in experiment planning, data analysis, and composition of manuscripts.*
Funding Support:	CDMRP, OCRF, MSKCC Cycle for Survival

Name:	*Jacob Ricca*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*10*
Contribution to Project:	*Mr. Ricca performed animal experiments, including survival experiments and isolation of tumor-infiltrating lymphocytes. Mr. Ricca in addition was involved in analysis of tumor tissues from patients with SCCOHT.*
Funding Support:	MSKCC Cycle for Survival

b.

Name:	*Anton Oseledchyk*
Project Role:	*Research Fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Oseledchyk performed animal experiments, as well as was involved in analysis of clinical data from the ovarian cancer patients treated with immunotherapy.*
Funding Support:	MSKCC Cycle for Survival

c. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

i. PI received additional funding support from OCRF. Funding support from Cycle for Survival has been completed.

ii. Changes in funding support do not change the level of effort for the project that is subject of the project report. Additional support from Cycle for Survival and now OCRF is subsumed toward the effort of the OCA award. The funding goes towards the coverage of consumables for the project and salary support of a technician working on the project.

d. **What other organizations were involved as partners?**

i. SCCOHT project was performed in collaboration with Dr. Doug Levine at New York University in New York, NY. The project was started when Dr. Levine was still at MSKCC. Our partnership with Dr. Levine has been limited to scientific collaboration; with parts of the project performed in his laboratory and part in ours. There were no personnel exhanges.

5. **SPECIAL REPORTING REQUIREMENTS**

a. **COLLABORATIVE AWARDS:**

Not applicable

b. **QUAD CHARTS:**

Not applicable

6. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

Abstracts submitted to the 2018 SGO meeting

1. Immune-reactive microenvironment of small cell carcinoma of the ovary, hypercalcemic type provides a rationale for evaluating immunotherapies to treat this malignancy

P. Jelinic[a], J. Ricca[b], E. Van Oudenhove[a], N. Olvera[a], T. Merghoub[b], D.A. Levine[a] and **D. Zamarin[b]**. *[a]NYU Langone Health, New York, NY, USA, [b]Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Objectives: Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare and very aggressive malignancy affecting very young women. The treatment options are limited and survival rates remain poor. Several SCCOHT patients have received anti-programmed death 1 (PD-1) immunotherapy with promising responses. Immunotherapies have been proven to be effective particularly in cancers that are hypermutated. Given that SCCOHT is a monogenic disease (SMARCA4 mutation is the sole alteration), SCCOHT patient's positive responses to immunotherapies is unusual. SCCOHT has not been immunoprofiled to date. Our objective was to characterize SCCOHT's immune landscape and to establish a rationale for using immunotherapies to treat SCCOHT patients.

Methods: We measured the expression of PD-L1, CD3 (T cells) and CD68 (macrophages) with immunofluorescence in eleven SCCOHT cases. SMARCA4 mutation status in SCCOHT was confirmed by MSK-IMPACT and immunohistochemistry. Immune-related gene expression profiling was performed with NanoString's nConter PanCancer Immunoprofiling panel.

Results: PD-L1 expression and T cell infiltration were detected in most tumors. PD-L1 expression was detected in both tumor and stromal cells. The majority of tumors were also infiltrated by macrophages. There was a strong association between T cell infiltration and PD-L1 expression. Gene expression profiling revealed that the high-PD-L1 group had increased PD-1 expression, as expected upon immune checkpoint activation. All immune cell types were elevated in the high- versus low-PD-L1 group, further demonstrating a correlation between PD-L1 upregulation and immune response. Cytolytic and antigen-presenting genes were also highly expressed in the high-PD-L1 group, suggesting an association between PD-L1 expression and high immune reactivity.

Conclusions: These data suggest that SCCOHT is an immunogenic malignancy with elevated PD-L1 expression and T cell infiltration. Our findings also highlight that mutational burden is not detrimental for tumor immunogenicity; SMARCA4 loss alone may promote immune recognition of SCCOHT. Our work provides a strong rationale for evaluating immunotherapies in SCCOHT patients.

2. Predictors of early treatment discontinuation in ovarian cancer patients on checkpoint blockade immunotherapy
J. Boland[a], M. Martin[a], N. Zecca[a], A. Iasonos[a], Q. Zhou[a], C.A. Aghajanian[a,b], P. Sabbatini[a,b], K.A. Cadoo[a,b] and **D. Zamarin**[a,b]. *[a]Memorial Sloan Kettering Cancer Center, New York, NY, USA, [b]Weill Cornell Medical College, New York, NY, USA*

Objectives: We sought to determine whether pre-treatment clinical biomarkers could predict early treatment discontinuation of ovarian cancer patients on checkpoint blockade immunotherapy agents.

Methods: A retrospective analysis was performed on all patients (pts) diagnosed with epithelial ovarian, primary peritoneal and fallopian tube cancer who were treated with checkpoint blockade immunotherapy at Memorial Sloan Kettering Cancer Center from January 1, 2006 to May 20, 2017. Pre-treatment clinical properties were recorded from the electronic medical record. Pre-treatment computed tomography scans were reviewed to assess for extent and sites of disease. Bulky disease was defined as having any lesion greater than or equal to 5 centimeters. Univariate logistic regression and a multivariate logistic model were built based on relevant clinical variables.

Results: Of 115 identified pts, 12 pts were excluded for discontinuation early due to toxicity to yield a cohort of N=103. 59.2% of patients (N=61) were discontinued early due to radiographic or symptomatic disease progression, with a median time on therapy of 10 weeks. On univariate analysis, pre-treatment bulky disease (p=0.038, OR 2.927), liver parenchymal metastases (p=0.009, OR 4.174), and bone metastases (p=0.045, OR 4.896) were predictive of early discontinuation, as shown in Table 1. On multivariate logistic analyses, the presence of liver parenchymal metastases was predictive of early discontinuation (p=0.024, OR 3.547). Symptomatic clinical progression was the reason for early discontinuation in 25 (41%) of the patients.

Conclusions: The retrospective analysis shows that ovarian cancer pts with bulky disease, liver parenchymal metastases, and bone metastases were more likely to discontinue early on checkpoint blockade immunotherapy. Given the potential for delayed responses to immunotherapy agents, pts who are at risk for early discontinuation due to clinical progression may not be suitable candidates for immunotherapy clinical trials. These findings could help guide the selection of appropriate patients that would be more likely to stay on trial beyond 12 weeks, in order to allow for the assessment of potential delayed benefit from these drugs.

3. Role of serum CA-125 in monitoring ovarian cancer patients on checkpoint blockade immunotherapy

Julia L. Boland[1], Qin Zhou[2], Alexia E. Iasonos PhD[2], Paul Sabbatini, MD[1,3], Carol Aghajanian, MD[1,3], **Dmitriy Zamarin, MD, PhD[1,3*], Karen A. Cadoo, MD[1,3*]**

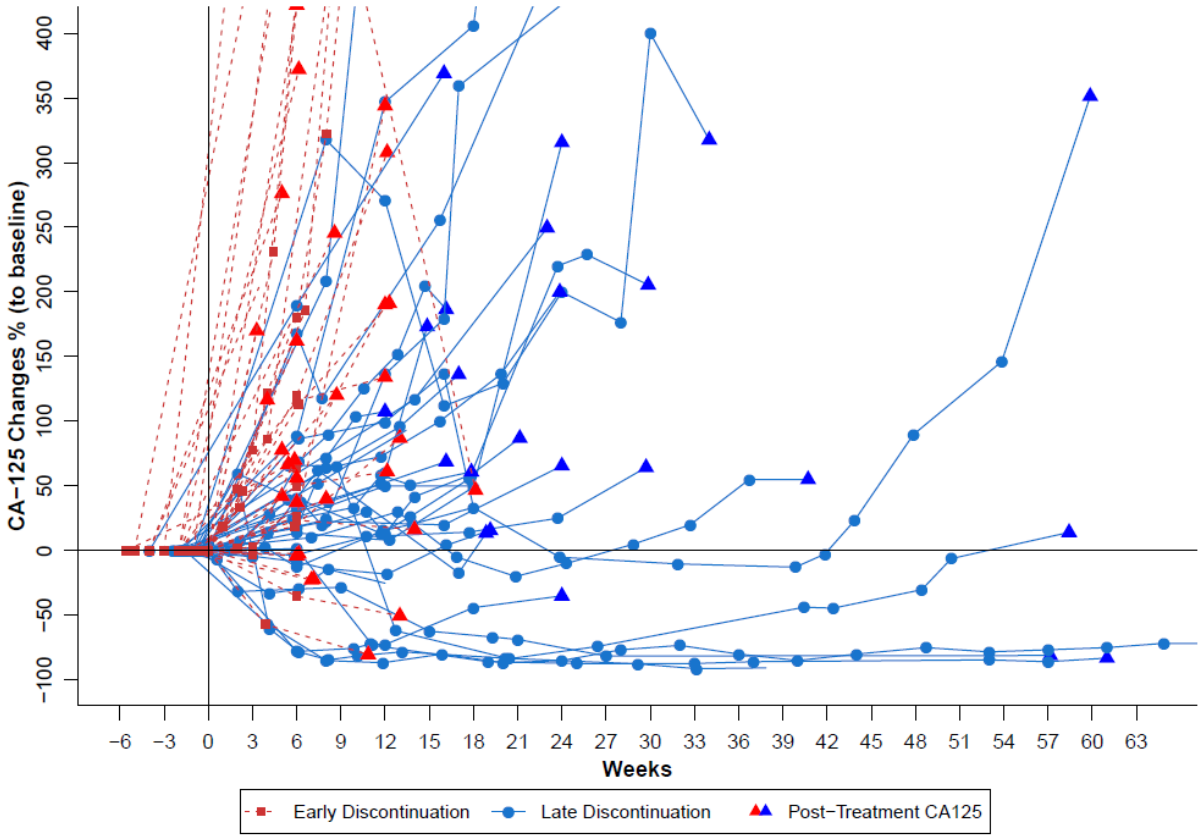
1. Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
2. Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA
3. Memorial Sloan Kettering Cancer Center and Weil Cornell Medical College, New York, NY, USA
*Co-mentors

Objectives: This study aims to evaluate whether serum cancer antigen-125 (CA-125) levels have utility in monitoring patients on checkpoint blockade immunotherapy.

Methods: A retrospective analysis was conducted on patients (pts) diagnosed with epithelial ovarian, primary peritoneal and fallopian tube cancer treated with checkpoint blockade immunotherapy at Memorial Sloan Kettering Cancer Center from Jan 2006 - May 2017. CA-125 levels (pre-, on-, and post-treatment) were recorded from the electronic medical record (EMR). CA-125 measurements were not uniform or per-protocol but were conducted at the discretion of the treating physician. The CA-125 measurement window was from 0 to 42 days before treatment start date and after treatment end date for pre- and post-treatment values, respectively. Pts whose treatment was discontinued prior to 12 weeks for clinical and/or radiographic progression were considered to have undergone early discontinuation. Means and medians of CA-125 in patients with early vs. late discontinuation were analyzed. Wilcoxon rank-sum tests were performed.

Results: Of 115 identified pts, 12 (10%) were excluded due to early discontinuation for toxicity. Of the remaining 72 pts who had baseline CA-125 measurement (median time of measurement 5 days before treatment start date, range 0-39 days before treatment start date), (figure 1), 51 (50%) had at least one CA-125 value on therapy, measured within 8 weeks of start (median time of measurement 42 days after treatment start date, range 7-56 days). 22 (43%) of these had early treatment discontinuation; 19 pts (86%) had increased CA-125 on therapy, however in 3 pts (14%), CA-125 declined. Conversely, in the 29 pts who did not experience early treatment discontinuation, while 8 pts (28%) had CA-125 decline, in 21 pts (72%) it increased. There was no statistically significant difference in the % change of CA-125 from baseline in the group who had early treatment discontinuation compared with those who did not. (Wilcoxon rank-sum test p=0.117)

Conclusions: Our analysis fails to distinguish a difference in the trend of CA-125 levels among pts who discontinue early versus pts who discontinue late on immunotherapy. The data suggests that physicians should apply caution when using CA-125 data to guide treatment decisions for patients on checkpoint blockade immunotherapy.


CA−125 Changes % (to baseline)
−100
−50
0
50
100
150
200
250
300
350
400
−6
−3
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
60
63
Weeks
Early Discontinuation
Late Discontinuation
Post−Treatment CA125

4. A phase 2 trial of TPIV200 (a poly-peptide vaccine against folate receptor alpha) plus durvalumab (anti-PD-L1 antibody) in patients with platinum resistant ovarian cancer.

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Introduction: Effective immunotherapeutic strategies are needed for ovarian cancer (OC). Immune checkpoint inhibitors have only modest benefit, perhaps in part due to the low immunogenicity of OC, associated with low mutation burden. Vaccination against tumor associated antigens (TAA's) is a potential strategy to increase therapeutic efficacy by enhancing immunogenicity. Folate Receptor alpha (FRα) is a TAA that is overexpressed by most OC's, and is a candidate immune target. TPIV200 is a multi-epitope anti-FRα vaccine that has been shown in a phase I trial to induce FRα-specific T cell activation. We hypothesized that combination therapy of TPIV200 with durvalumab will be safe and efficacious in patients with OC.

Objectives: The primary endpoint (EP) of this study was assessment of overall response rate. The co-primary EP was progression free survival at 6 months. Secondary EP's evaluated in the study were safety and tolerability of the combination, and disease control rate. Exploratory correlative EP's included expression of PD-L1 and FRα, as well as both tissue and circulating immune signatures.

Methods: This phase II clinical trial was based on a Simon two stage design. Patients with platinum resistant or refractory OC were enrolled over a 10-month period. Patients were treated with TPIV200 and GM-CSF on day 1 of cycles 1-6 and durvalumab on days 1 and 15 of cycles 1-12. Radiologic assessments were conducted every 3 cycles. Treatment was continued until evidence of clinical or radiologic progression, intolerance, or withdrawal. Neither PD-L1 nor FRα expression was required. There was no limit on prior therapy, 14 (14/27) patients had < 3 prior treatments and 13 (13/27) had > 3 priors.

Results: Between 6/2016- 4/2017, 27 women between ages 42-76 (median age 64) were enrolled. Of these patients, 85% (23/27) had high grade serous OC. The remainder had clear cell (2), endometrioid (1), and mixed (1) histologies. There were no grade 3 or 4 treatment-related adverse events (AEs). There were 2 grade 2 immune related AE's in the form of autoimmune Type I diabetes and pneumonitis. Both patients were successfully managed with insulin and drug discontinuation, respectively.

Conclusions: TPIV200/huFR-1 and durvalumab can be safely combined in heavily-pretreated patients with platinum-resistant refractory OC. Analysis for the primary EP is ongoing and will be presented.